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Short communication

Detection of multiple viruses in queens of the honey bee *Apis mellifera* L. ☆

Yanping Chen *, Jeffery S. Pettis, Mark F. Feldlaufer

USDA, Agricultural Research Service, Bee Research Laboratory, Building 476; BARC-East, Beltsville, MD 20705, USA

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Abstract

Individual honey bee *Apis mellifera* L. queens were examined for the presence of six honey bee viruses including acute bee paralysis virus, chronic bee paralysis virus, black queen cell virus, deformed wing virus, Kashmir bee virus, and sacbrood virus. All viruses, except ABPV, were detected in the samples. Among queens examined for virus infections, 93% had multiple virus infections. The detection of viruses in queens raises the possibility of a vertical transmission pathway wherein infected queens can pass virus through their eggs to their offspring.
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Keywords: *Apis mellifera*; Queen; Viruses; Single infection; Multiple infections; Vertical transmission

The honey bee *Apis mellifera* L. has been reported to harbor at least 18 viruses (Allen and Ball, 1996; Bailey and Ball, 1991). Among viruses infecting honey bees, 10 have been found in the United States (Allen and Ball, 1996). Except for filamentous bee virus, all honey bee viruses reported so far are single stranded RNA viruses 20–30 nm in diameter, isometrically shaped, nonoccluded, possessing a buoyant density in CsCl ranging from 1.33 to 1.42 g/ml, and a 100–190S sedimentation coefficient (Bailey, 1976). Hence, they are very difficult to distinguish using physical characteristics. Furthermore, most bee viruses persist as inapparent infections and cause no overt signs of disease (Bailey, 1976), it is very difficult to identify bee virus infections and almost impossible to differentiate mixed virus infections based only on the field observation. The development of molecular technologies, however, has provided a powerful tool for specific, sensitive, and rapid identification of bee viruses. The availability of complete genome sequences of five viruses, acute bee paralysis virus (ABPV; GenBank Accession No. AF150629), black queen cell virus (BQCV; GenBank Accession No. AF183905), deformed wing virus (DWV; GenBank Accession No. NC004830), Kashmir bee virus (KBV; GenBank Accession No. NC004807), and sacbrood virus (SBV; GenBank Accession No. AF092924) and partial genome sequences of chronic bee paralysis virus (CBPV; GenBank Accession No. AF461061) makes the molecular detection and characterization of these viruses possible (Bakonyi et al., 2002; Benjeddou et al., 2001; Chen et al., 2004c; Evans, 2001; Genersch, 2005; Grabenstiner et al., 2001; Hung et al., 1996; Ribiere et al., 2002; Stoltz et al., 1995; Tentcheva et al., 2004). Utilizing RT-PCR methods, viruses have been detected in different life stages of bees as well as in the parasitic mite *Varroa destructor* (Chen et al., 2005, 2004a). However, despite the increasing knowledge of bee virus infections, there is little information regarding the virus status of the honey bee queens. In this report, we examine the virus status of individual queen bees.

Twenty-nine queens from honey bee colonies maintained in Beltsville, MD and Sapelo Island, GA were used in this study. Queens from GA were collected in centrifuge tubes on

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* Corresponding author. Fax: +1 301 504 8736.
E-mail address: chenj@ba.ars.usda.gov (Y. Chen).

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dry ice and shipped overnight to MD for analyses. Total RNA was extracted from individual queens using an RNA isolation kit (TRIzol; Invitrogen; Carlsbad, CA) according to the manufacturer's protocol. RNA samples were dissolved in DEPC-treated water in the presence of ribonuclease inhibitor (Invitrogen) and stored at −80 °C freezer until used. Total RNA samples extracted from individual queens were examined for the presence of six bee viruses (ABPV, BQCV, CBPV, DWV, KBV, and SBV) by RT-PCR assay (Access RT-PCR system; Promega; Madison, WI). The primers used are shown in Table 1. The reaction mixture and RT-PCR thermal cycling profiles were performed as previously described (Chen et al., 2004a,b). Negative (water) and positive controls (previously identified positive sample) were included in each run of the RT-PCR reaction. PCR products were electrophoresed in 1% agarose gel containing 0.5 ug/ml ethidium bromide and visualized under UV light. To prevent possible contamination of PCR products, the RT-PCR reaction mixture was prepared in a PCR chamber (PLAS, Lansing, MI) and the gel electrophoresis was conducted in a different room. PCR bands specific for each virus were purified using Wizard PCR Prep DNA Purification System (Promega, Madison, WI) and sequenced to confirm the specificity of RT-PCR assay. The nucleotide sequences of PCR products were analyzed and compared with sequences published at the GenBank, National Center for Biotechnology Information, NIH.

This study demonstrates that adult queen bees can harbor multiple viruses. Although no obvious disease was observed in the queens, all viruses, except ABPV were detected in the samples (Fig. 1). Sequence analyses indicated that RT-PCR bands representing BQCV, CPBV, DWV, KBV, and SBV were virus specific. Among the queens ($n = 29$), 86% were positive for BQCV, 14% were positive for CBPV, 100% were positive for DWV, 21% were positive for KBV, and 62% were positive for SBV. The results also indicated that queens could be co-infected with multiple viruses, simultaneously. Of the 29 queens examined, 93% had multiple virus infections of two, three, four or five viruses

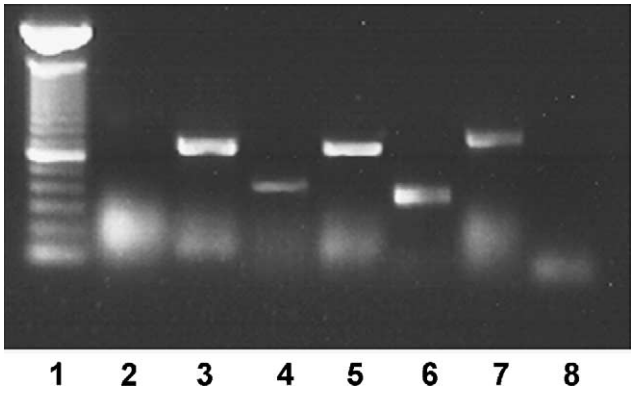

Fig. 1. RT-PCR results of a representative queen. Total RNA extracted from the queen was examined for the presence of six viruses, ABPV, BQCV, CBPV, DWV, KBV, and SBV by RT-PCR individually. Primer pair specific for ABPV, BQCV, CBPV, DWV, KBV, and SBV amplified a PCR fragment of 900, 700, 455, 702, 415, and 824 bp, respectively. Except for ABPV, other five viruses were detected in the RNA sample of the queen. Lane 1, 100 bp DNA ladder; lane 2, ABPV; lane 3, BQCV; lane 4, CBPV; lane 5 DWV; lane 6, KBV; lane 7, SBV; lane 8 negative control (H_2O).

(Table 2). Although these results demonstrate that viruses can occur in honey bee queens, it is not clear how these infections affect the behavior and/or physiology of the queens. Further studies will be necessary to determine if one or more of these viruses have a deleterious effect.

Our previous research with DWV revealed that this virus could be detected in different stages of honey bees, including adults, pupae, larvae, and eggs as well as in the mite *V. destructor* (Chen et al., 2005). Detection of DWV in these parasitic mites supports the previous finding that *V. destructor* is involved in the transmission of this virus (Bowen-Walker et al., 1999). However, our detection of DWV in honey bee eggs and in larval stages that are not normally associated with mite infestation led us to postulate another route of transmission, i.e., that infected queens in a colony might transmit virus vertically to the eggs. The detection of virus infection in immature stages has also been reported in the red imported fire ant, *Solenopsis*

Table 1
Primers used in the study

Virus	Primers	Product size (bp)	References
ABPV	ABPV-F: (5-ttatgtgtccagagactgtatcca-3′) ABPV-R: (5′-gctcctattgctcggtttttcggt-3′)	900	Benjeddou et al. (2001)
CBPV	CBPV-F: (5′-agttgtcatggttaacaggatacgag-3′) CBPV-R: (5′-tctaatcttagcacgaaagccgag-3′)	455	Ribiere et al. (2002)
BQCV	BQCV-F: (5′-tggtcagctcccactaccttaaac-3′) BQCV-R: (5′-gcaacaagaagaaacgtaaaccac-3′)	700	Benjeddou et al. (2001)
DWV	DWV-F: (5′-atcagcgcttagtggaggaa-3′) DWV-R: (5′-tcgacaattttcggacatca-3′)	702	Chen et al. (2004b)
KBV	KBV-F: (5′-gatgaacgtcgacctattga-3′) KBV-R: (5′-tgtgggttggctatgagtca-3′)	415	Stoltz et al. (1995)
SBV	SBV-F: (5′-gctgaggtaggatctttgcgt-3′) SBV-R: (5′-tcatcatcttcaccatccga-3′)	824	Chen et al. (2004c)

Table 2
Virus infection in Queens of honey bees

Queen	Virus						Multiple (M) or Single (S) Infection
	ABPV	BQCV	CBPV	DWV	KBV	SBV	
1	–	+	–	+	–	+	M
2	–	+	–	+	–	+	M
3	–	+	–	+	–	+	M
4	–	+	–	+	–	+	M
5	–	+	–	+	–	+	M
6	–	+	–	+	+	–	M
7	–	–	–	+	–	–	S
8	–	–	–	+	–	+	M
9	–	+	+	+	–	+	M
10	–	–	+	+	+	–	M
11	–	+	–	+	–	–	M
12	–	+	–	+	+	–	M
13	–	+	–	+	–	–	M
14	–	+	–	+	+	+	M
15	–	+	–	+	–	–	M
16	–	+	–	+	–	+	M
17	–	+	–	+	+	+	M
18	–	+	–	+	–	+	M
19	–	–	–	+	–	–	S
20	–	+	+	+	+	+	M
21	–	+	+	+	–	+	M
22	–	+	–	+	–	–	M
23	–	+	–	+	–	–	M
24	–	+	–	+	–	–	M
25	–	+	–	+	–	+	M
26	–	+	–	+	–	+	M
27	–	+	–	+	–	+	M
28	–	+	–	+	–	+	M
29	–	+	–	+	–	+	M
Total	0	86%	14%	100%	21%	62%	7% of S; 93% of M

invicta. In 2004, Valles et al., showed that SINV-1, a positive stranded RNA virus, attacked all developmental stages of the fire ant including the egg stage which implicated the vertical transmission of the virus. Our demonstration that queens can harbor multiple viruses is consistent with this hypothesis and we are currently investigating this possible vertical route of transmission.

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